PATENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 31 August 2001 (31.08.01)	
International application No. PCT/US00/40588	**Applicant's or agent's file reference** 3051-66796
International filing date (day/month/year) 07 August 2000 (07.08.00)	**Priority date** (day/month/year) 09 August 1999 (09.08.99)
Applicant WOOD, Alastair, J., J. et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   01 March 2001 (01.03.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

10/089,827

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
3051-66796		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/40588	07 AUGUST 2000	09 AUGUST 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant VANDERBILT UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01 MARCH 2001	31 OCTOBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Jake Bridges RUSSELL TRAVERS
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/40588

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-17 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 18-25 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-6 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/40588

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 27-28

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 27-28 are so unclear that no meaningful opinion could be formed *(specify)*.

Claims 27 and 26 are drafted as improper multiple dependant claims, thus, failing to meet those criteria set forth in the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 27-28 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/40588

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-26, 29-36	YES
	Claims	none	NO
Inventive Step (IS)	Claims	1-26, 29-36	YES
	Claims	none	NO
Industrial Applicability (IA)	Claims	1-26, 29-36	YES
	Claims	none	NO

2. citations and explanations (Rule 70.7)

Claims 1-26 and 29-36 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest employing the disclosed tricyclic compounds herein claimed for the recited antiviral use.

NEW CITATIONS

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A61K 31/495, 31/50, 31/205, 31/24, 31/22, 31/195, 31/20 and US Cl.: 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568

Express Mail No.: EL230048285US

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: STEVEN R. LAMMERT
BARNES & THORNBURG
11 SOUTH MERIDIAN STREET
INDIANAPOLIS, IN 46204

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing *(day/month/year)* 26 MAR 2001
Applicant's or agent's file reference 3051-66796	**FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US00/40588	International filing date *(day/month/year)* 07 AUGUST 2000
Applicant VANDERBILT UNIVERSITY	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RUSSELL TRAVERS Telephone No. (703) 308-1235

(See notes on accompanying sheet)

Form PCT/ISA/220 (July 1998)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 3051-66796	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US00/40588	International filing date *(day/month/year)* 07 AUGUST 2000	(Earliest) Priority Date *(day/month/year)* 09 AUGUST 1999
Applicant VANDERBILT UNIVERSITY		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readable form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/40588

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 27-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/40588

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/495, 31/50, 31/205, 31/24, 31/22, 31/195, 31/20

US CL : 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: compounds and anticancer therapy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,939,456 A (PERRINE) 17 August 1999, see entire document	1-26, 29-36
A	US 5,643,909 A (PFISTER et al.) 01 July 1997, see entire document	1-26, 29-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 2001	Date of mailing of the international search report 26 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RUSSELL TRAVERS Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)★

Express Mail No.: EL230048285US

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 3051-66796

Box No. I TITLE OF INVENTION

ANTIVIRAL THERAPY USE OF P_GLYCOPROTEIN MODULATORS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VANDERBILT UNIVERSITY
305 Kirkland Hall
Nashville, TN 37240
US

☐ This person is also inventor.

Telephone No. (615) 343-2430

Facsimile No. (615) 343-4419

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WOOD, Alastair J. J.
P.O. Box 159319
Nashville, TN 37215-9319
US

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LAMMERT, Steven R.
BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

Telephone No. (317) 236-1313

Facsimile No. (317) 231-7433

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

KIM, Richard B.
5101 Fredericksburg Way East
Brentwood, TN 37027
US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CA

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WILKINSON, Grant R.
612 Valley Trace Court
Nashville, TN 37221-3123
US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes, at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AG Antigua and Barbuda
☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ BZ Belize
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China .....
☒ CR Costa Rica .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ DM Dominica
☒ DZ Algeria .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco .....
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ MZ Mozambique
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone .....
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ TZ United Republic of Tanzania
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZA South Africa .....
☒ ZW Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

See Notes to the request form

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (09.08.99) 09 August 1999	09/370,266	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen, the two-letter code may be used):* ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	4
description (excluding sequence listing part)	17
claims	8
abstract	1
drawings	2
sequence listing part of description	0
Total number of sheets	32

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney (4 original PCT General Power of Attorney forms with 1 Delegation of Authority
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter to the US/RO Return Postal Card

Figure of the drawings which should accompany the abstract: None

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Jill T. Powlick, Agent for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

VANDERBILT UNIVERSITY
305 Kirkland Hall
Nashville, TN 37240
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

LAMMERT, Steven R.; COFFEY, William R.; CONARD, Richard D.; REZEK, Richard A.; HARRISON, Nancy, J.; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; STEIN, Arland T.; RICHARDS, William B.; HAIGH, Christopher E.; SWEENEY, James R. II; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; MARTIN, Alice O.; COOPER, Gregory S.; All Appointed Agents of the Address:

BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power)*

By: Janis Elsner
Janis Elsner, Associate Director

(20.07.00)
Date: 20 July 2000
Day/ Month/ Year

Form PCT/Model of general power of attorney (for several international applications) (July 1992)

DELEGATION OF AUTHORITY

Pursuant to the authority delegated to me by resolution of the Executive Committee of the Board of Trust adopted on December 11, 1990, I delegate to Janis Elsner, Associate Director, Office of Technology Transfer, the authority to execute on behalf of Vanderbilt University, license agreements relating to technology owned by Vanderbilt, including agreements to create new business entities in which Vanderbilt will become an equity owner, agreements granting an option to a party to negotiate a license agreement, confidentiality agreements relating to technology disclosed to the Office of Technology Transfer and letters of intent to enter into licensing negotiations. Before licenses, options or letters of intent are executed by Ms. Elsner, appropriate University administrators, the creators of the particular technology, and a representative of the Office of General Counsel shall review and approve the terms of the proposed license, option or letter of intent. On a monthly basis, Ms. Elsner shall forward to me summaries of licenses, options and letters of intent that have been executed following the aforementioned approvals.

In addition to the foregoing, and also pursuant to the authority delegated to me by the resolution of the Executive Committee of the Board of Trust adopted on December 11, 1990, I delegate to Ms. Elsner authority to execute on behalf of Vanderbilt University all documents regarding technology owned by Vanderbilt and required to be filed in the U. S. Patent and Trademark Office and U. S. Copyrights Office, or equivalent foreign governmental bodies, in connection with intellectual property rights of Vanderbilt.

Jeff Carr, Vice-Chancellor for University Relations and General Counsel

1/26/00

Date

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

WOOD, Alastair J. J.
P.O. Box 159319
Nashville, TN 37215-9319
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)
LAMMERT, Steven R.; COFFEY, William R.; CONARD, Richard D.; REZEK, Richard A.; HARRISON, Nancy, J.; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; STEIN, Arland T.; RICHARDS, William B.; HAIGH, Christopher E.; SWEENEY, James R. II; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; MARTIN, Alice O.; COOPER, Gregory S.; All Appointed Agents of the Address:
BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power).*

Alastair J. J. WOOD

Date: 26/07/07.
Day/ Month/ Year

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

KIM, Richard B.
5101 Fredericksburg Way East
Brentwood, TN 37027
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)
LAMMERT, Steven R.; COFFEY, William R.; CONARD, Richard D.; REZEK, Richard A.; HARRISON, Nancy, J.; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; STEIN, Arland T.; RICHARDS, William B.; HAIGH, Christopher E.; SWEENEY, James R. II; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; MARTIN, Alice O.; COOPER, Gregory S.; All Appointed Agents of the Address:

BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power)*

Richard B. KIM

Date: 21 07 2000
Day/ Month/ Year

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

WILKINSON, Grant R.
612 Valley Trace Court
Nashville, TN 37221-3123
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)
LAMMERT, Steven R.; COFFEY, William R.; CONARD, Richard D.; REZEK, Richard A.; HARRISON, Nancy, J.; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; STEIN, Arland T.; RICHARDS, William B.; HAIGH, Christopher E.; SWEENEY, James R. II; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; MARTIN, Alice O.; COOPER, Gregory S.; All Appointed Agents of the Address:

BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign. next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power)*

Grant R. WILKINSON

Date: 24/7/00
Day/ Month/ Year

Express Mail No.: EL230048285US

M

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

RECEIVED
NOV 13 2001
BARNES & THORNBURG

To: STEVEN R. LAMMERT
BARNES & THORNBURG
11 SOUTH MERIDIAN STREET
INDIANAPOLIS, IN 46204

DOCKETED
FOR 1ST. Written Opinion
BY
DATE 11/14/01
CHE'D BY
DATE

PCT

WRITTEN OPINION

(PCT Rule 66)

	Date of Mailing *(day/month/year)* 08 NOV 2001
Applicant's or agent's file reference 3051-66796	REPLY DUE within ONE months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/40588	07 AUGUST 2000	09 AUGUST 1999

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

Applicant
VANDERBILT UNIVERSITY

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is 09 DECEMBER 2001

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer RUSSELL TRAVERS
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/408 (cover sheet) (July 1998) ★

WRIT OPINION

International application No.
PCT/S00/40588

I. Basis of the opinion

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-17, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 18-25, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages 1-6, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

WRIT[redacted]OPINION

International application No.
P[redacted]S00/40588

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 27-28

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 27-28.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard

☐ the computer readable form has not been furnished or does not comply with the standard

WRITTEN OPINION

International application No.
PCT/US00/40588

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-26, 29-36	YES
	Claims	none	NO
Inventive Step (IS)	Claims	1-26, 29-36	YES
	Claims	none	NO
Industrial Applicability (IA)	Claims	1-26, 29-36	YES
	Claims	none	NO

2. citations and explanations

Claims 1-26 and 29-36 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest employing the disclosed tricyclic compounds herein claimed for the recited antiviral use.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): A61K 31/495, 31/50, 31/205, 31/24, 31/22, 31/195, 31/20 and US Cl.: 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/10387 A3

(51) International Patent Classification[7]: A61K 31/495, 31/50, 31/205, 31/24, 31/22, 31/195, 31/20

(21) International Application Number: PCT/US00/40588

(22) International Filing Date: 7 August 2000 (07.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/370,266 9 August 1999 (09.08.1999) US

(71) Applicant *(for all designated States except US)*: **VANDERBILT UNIVERSITY** [US/US]; 305 Kirkland Hall, Nashville, TN 37240 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WOOD, Alastair, J., J.** [US/US]; P.O. Box 159319, Nashville, TN 37215-9319 (US). **KIM, Richard, B.** [CA/US]; 5101 Fredericksburg Way East, Brentwood, TN 37027 (US). **WILKINSON, Grant, R.** [US/US]; 612 Valley Trace Court, Nashville, TN 37221-3123 (US).

(74) Agent: **LAMMERT, Steven, R.**; Barnes & Thornburg, 11 South Meridian Street, Indianapolis, IN 46204 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
23 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/10387 A3

(54) Title: ANTIVIRAL THERAPY USE OF P-GLYCOPROTEIN MODULATORS

(57) Abstract: The present invention relates to a pharmaceutical composition comprising a 10, 11 methanodibenzosuberane and use thereof for the treatment of HIV infection. Co-administration of the 10, 11 methanodibenzosuberane with an HIV protease inhibitor increases the concentration of the protease inhibitor in certain tissues, including the brain and testes, without substantially increasing plasma levels of the protease inhibitor. Accordingly, additional antiviral therapy can be achieved without use of increased drug dosages, thereby reducing the potential for occurrence of undesirable side effects deriving from drug toxicity.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/40588

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/495, 31/50, 31/205, 31/24, 31/22, 31/195, 31/20
US CL : 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/252.12, 252.13, 253.01, 554, 538, 546, 563, 568

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: compounds and anticancer therapy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,939,456 A (PERRINE) 17 August 1999, see entire document	1-26, 29-36
A	US 5,643,909 A (PFISTER et al.) 01 July 1997, see entire document	1-26, 29-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 2001	Date of mailing of the international search report 26 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RUSSELL TRAVERS Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/40588

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 27-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2001 (15.02.2001)

PCT

(10) International Publication Number
WO 01/10387 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US00/40588

(22) International Filing Date: 7 August 2000 (07.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/370,266 9 August 1999 (09.08.1999) US

(71) Applicant *(for all designated States except US)*: **VANDERBILT UNIVERSITY** [US/US]; 305 Kirkland Hall, Nashville, TN 37240 (US).

(72) Inventors; and
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(54) Title: ANTIVIRAL THERAPY USE OF P-GLYCOPROTEIN MODULATORS

(57) Abstract: The present invention relates to a pharmaceutical composition comprising a 10, 11 methanodibenzosuberane and use thereof for the treatment of HIV infection. Co-administration of the 10, 11 methanodibenzosuberane with an HIV protease inhibitor increases the concentration of the protease inhibitor in certain tissues, including the brain and testes, without substantially increasing plasma levels of the protease inhibitor. Accordingly, additional antiviral therapy can be achieved without use of increased drug dosages, thereby reducing the potential for occurrence of undesirable side effects deriving from drug toxicity.

ANTIVIRAL THERAPY USE OF P-GLYCOPROTEIN MODULATORS

Field of the Invention

The present invention relates to treatment of viral infections. More particularly the present invention is directed to the use of certain P-glycoprotein modulators to increase the concentration of HIV-protease inhibitors in certain tissues.

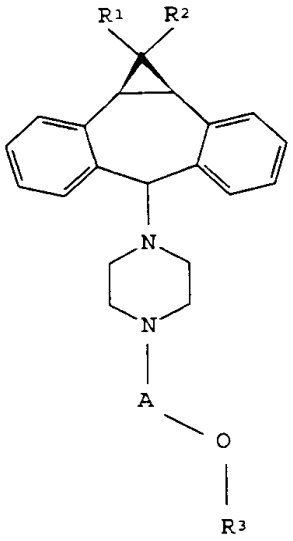
Background and Summary of the Invention

HIV protease inhibitors have proven to be effective in the treatment of HIV-1 infection. However, the utility of such drugs can be limited due to poor transport across certain biological membranes. Oral absorption of protease inhibitors is often low and variable, and penetration into certain tissues, including the brain and testes, is often poor. The resultant non-uniform distribution of the antiviral drug in the body leaves certain tissues as sanctuaries for viral proliferation.

P-glycoprotein is an ATP dependent efflux membrane transporter with broad substrate specificity for a variety of structurally diverse drugs. P-glycoprotein is distributed in various normal tissues, including, of particular importance in drug disposition, epithelial cells in the gastrointestinal tract, the liver, and the kidney. Apical expression of P-glycoprotein in such tissues results in reduced absorption (gastrointestinal tract), and enhanced elimination into the bile (liver) and urine (kidney) for drugs functioning as P-glycoprotein substrates. In addition, expression of P-glycoprotein at the level of the blood-brain barrier has been shown to be a critical factor in preventing the entry of some drugs into the central nervous system. Previous work has shown that various HIV-1 protease inhibitors are substrates of P-glycoprotein, explaining some of the limits on membrane permeability of these drugs. See, for example, Kim, R.B., et al., The Drug Transporter P-Glycoprotein Limits Oral Absorption and Brain Entry of HIV-1 Protease Inhibitors, J. Clin. Invest., 101:289-294, 1998.

Certain 10,11-methanodibenzosuberane derivatives have been shown to be pharmaceutically active agents in the treatment of multidrug resistance in cancer therapy. See, for example, U.S. Patents Nos. 5,654,304 and 5,874,434. Such compounds are known to interact with P-glycoprotein.

The present invention relates to a use of a 10,11-methanodibenzosuberanes of formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

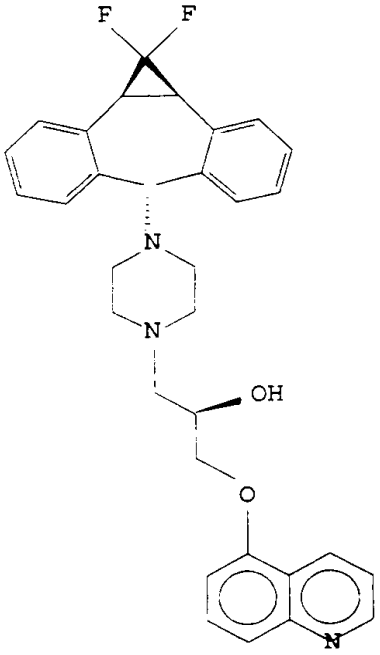
R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof; for the manufacture of a medicament for the treatment of HIV in a patient undergoing treatment with an HIV protease inhibitor. The use increases the concentration of the HIV inhibitor in the brain and/or testes of the patient without significantly increasing plasma levels of the protease inhibitor. Accordingly, more effective antiviral therapy can be achieved without use of increased drug dosages, thereby reducing the potential for occurrence of undesirable side effects deriving from drug toxicity. Thus, one aspect of this invention relates to a method for increasing the concentration of an HIV protease inhibitor in the brain of a patient, the method comprising administering to an HIV infected patient an amount of a 10,11-methanodibenzosuberane of formula (I), or a pharmaceutically acceptable salt thereof, and co-administering to the patient a therapeutically effective amount of the protease inhibitor.

Another related aspect of this invention is a method of treatment of an HIV infected patient. The method comprises administering a compound comprising a 10,11-methanodibenzosuberane of formula (I) in an amount effective to increase the concentration of a co-administered protease inhibitor in the brain and testes of the patient.

In another embodiment, the 10,11-methanodibenzosuberane of formula (I) is administered in combination with a protease inhibitor to increase concentrations of the protease inhibitor in the brain.

Still another aspect of this invention is a pharmaceutical composition comprising a protease inhibitor, most preferably nelfinavir, and a 10,11-methanodibenzosuberane of formula (I), with a pharmaceutical carrier. In a preferred embodiment, the 10,11-methanodibenzosuberane is a compound of formula (II):

II



Still another aspect of this invention is the use of an HIV protease inhibitor for the manufacture of a medicament for the treatment of HIV wherein the concentration of the protease inhibitor in the brain is increased by co-administration with a 10, 11-methanodibenzosuberane of formula (I), or a pharmaceutically acceptable salt thereof.

Additional features of the present invention will become apparent to those skilled in the art upon consideration of the following detailed description of preferred embodiments exemplifying the best mode of carrying out the invention.

Brief Description of the Drawings

Fig. 1 is a plot of percent inhibition of P-glycoprotein mediated [^{3}H]-digoxin transport across a Caco-2 cell culture monolayer verses concentration of putative inhibitor at varying concentrations of formula (II) (◇), nelfinavir (●), ritonavir (○), saquinavir (■), and indinavir (△).

Fig. 2 shows tissue levels of [^{14}C]-nelfinavir in *mdrla* (+/+) mice given 50 mg/kg of formula (II) (plasma - open symbols, brain - closed symbols) in divided doses 30 min prior to and simultaneously with (5 mg/kg) [^{14}C]-nelfinavir (triangles) or vehicle (circles).

Fig. 3 shows the effect of P-glycoprotein inhibitors on tissue:plasma concentration ratios of [^{14}C]-nelfinavir in *mdrla* (+/+) and *mdrla* (-/-) mice.

Detailed Description of the Invention

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein. Additional details on the preparation of such compounds, and the meaning and scope of the terminology and definitions thereof, are detailed in U.S. Patent No. 5,654,304.

The term "lower acyloxy" refers to the group --O--C(O)--R' where R' is lower alkyl.

The term "heteroaryl" refers to a monovalent unsaturated aromatic carbocyclic radical having at least one hetero atom, such as N, O or S, within the ring, such as quinolyl, benzofuranyl and pyridyl.

A "pharmaceutically acceptable salt" may be any salt derived from an inorganic or organic acid. The term "pharmaceutically acceptable anion" refers to the anion of such acid addition salts. The salt and/or the anion are chosen not to be biologically or otherwise undesirable.

The term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not todevelop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

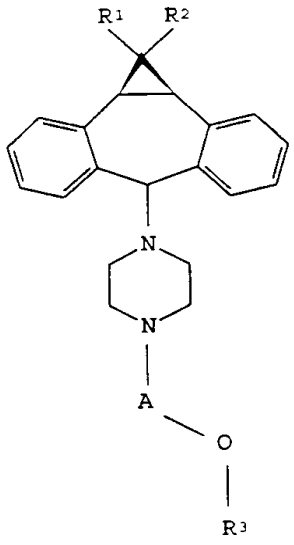
The term "effective amount" means a dosage sufficient to provide treatment for the disease state being treated. This will vary depending on the patient, the disease and the treatment being effected.

The term "co-administer" means the administration of more than one active agent as part of the same treatment regimen, whether they are administered simultaneously or at different times.

"Structure of formula (I)" refers to the generic structure of the compounds of the invention.

The present invention is a method for increasing the concentration of an HIV protease inhibitor in the brain and testes of a patient, said method comprising administering to an HIV-infected patient an amount of a 10,11-methanodibenzosuberanes of the formula (I):

I



wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

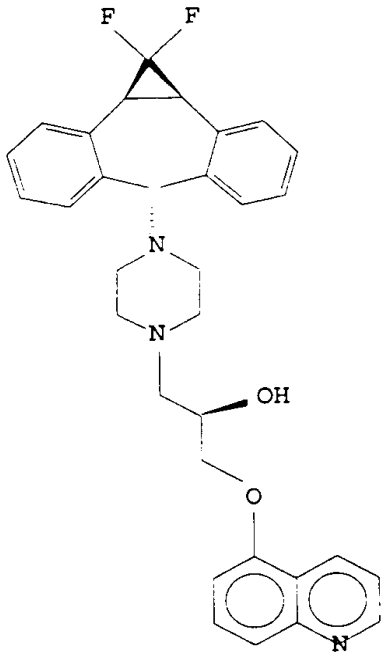
R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salts thereof; and co-administering to the patient a therapeutically effective amount to the protease inhibitor.

In a preferred embodiment, a compound of formula (I) is used wherein A is $-CH_2CHR^aCH_2-$. In another preferred embodiment, R^1 and R^2 are F. In still another preferred embodiment, R^3 is an optionally substituted quinolyl, preferably quinol-5-yl.

In another preferred embodiment of the present invention, a compound of formula (II):



II

is employed with protease inhibitors in the method of the present invention.

Examples of such protease inhibitors contemplated by the present invention are NELFINAVIR, which is preferably administered as the mesylate salt at

750 mg three times per day (Agouron Pharmaceuticals (La Jolla, CA)) (U.S. Patent No. 5,484,926); RITONAVIR, which is preferably administered at 600 mg twice daily (Roche Ltd. (Lewes, UK) (U.S. Patent No. 5,484,801); SAQUINAVIR, which is preferably administered as the mesylate salt at 1,200 mg three times per day (Roche Discovery (Rahway, NJ)) (U.S. Patent No. 5,196,438); INDINAVIR, which is preferably administered as the sulfate salt at 800 mg three times per day (Merck Research Laboratories) (U.S. Patent No. 5,413,999); and AMPRENAVIR, which is preferably administered at 1,200 mg twice daily (U.S. Patent No. 5,585,397). The skilled artisan would recognize that this list is not exhaustive. Additionally the skilled artisan would recognize that the protease inhibitor's administration to a patient may vary from the preferred.

The HIV-1 virus enters the brain and other organs such as the testes relatively early after primary infection. Reduction of the viral load in such organs has proven to be difficult, as most of the current HIV antiviral agents do not readily penetrate into the tissues to provide concentrations effective to prevent viral replication. See Groothius, D.R., and Levy, R.M., The entry of antiviral drugs into the central nervous system, J. NeuroVirology, 3:387-400, 1997. The low rate of drug transport into these pharmacologic sanctuary sites is the consequence of a functional barrier to drug entry. HIV protease inhibitors have been found to be excellent substrates for the membrane efflux pump P-glycoprotein, which is localized in the apical domain of capillary endothelial cells of the brain and testis. The P-glycoprotein pump works to limit drug distribution into these tissues. See, for example, Kim, R.B., et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest., 101:289-294, 1998; Lee, C.G.L., et al., HIV-1 protease inhibitors are substrates for the *mdrl* multidrug transporter, Biochemistry, 37:3594-3601, 1998; Kim, A.E., et al., Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein, J. Pharmaco. Exp. Ther., 286:1439-1445, 1998; Thiebaut, F., et al., Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissue, Proc. Natl. Acad. Sci., U.S.A., 84:7735-7738, 1987; Gordon-Cardo, C., et al., Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites, Proc. Natl. Acad. Sci., U.S.A., 86:695-698, 1989.

The present invention enables pharmacological inhibition of the functional activity of the P-glycoprotein transporter on HIV protease inhibitor substrates through use of a 10,11-methanodibenzosuberane of formula (I) co-administered with an HIV protease inhibitor. Such modulation of P-glycoprotein activity results in significantly enhanced HIV protease inhibitor concentrations in both the brain and testes relative to drug concentration in plasma.

The magnitude of the effect of P-glycoprotein inhibition attainable by administration of the compounds of formula (I) is tissue dependent; for example, the tissue:plasma drug concentration ratio is enhanced in the brain to a greater extent than in the testes. This difference is believed to be related to the level of P-glycoprotein function in the respective tissues. There is about a 30-fold difference in nelfinavir concentration in the brain of *mdrla*(+/+) *and mdrla*(-/-) mice compared to only a 4--fold concentration difference in the testes. P-glycoprotein inhibition using the compounds of formula (I) exhibits similar tissue differences. Notably, however, nelfinavir concentration differences achieved in both organs indicates a 75 to 90% absence of P-glycoprotein function based on comparable data in the *mdrla*(-/-) mice. At the highest doses of the compound of formula (II), the concentrations of nelfinavir in the brain and testes are equal to or higher than the drug concentration in plasma. Co-administration of a 10,11-methanodibenzosuberane of formula (I) with an HIV protease inhibitor in accordance with this invention minimizes P-glycoprotein modulated drug concentration differences between plasma and the brain and testes, thereby reducing or eliminating these tissues as sanctuaries for viral proliferation in patients receiving protease inhibitor therapy.

The present invention provides advantages over use of prior art P-glycoprotein inhibitors such as quinidine, verapamil, valspodar, and cyclosporine A, which are known to interact with drug metabolizing enzymes, in particular, members of the cytochrome P4503A subfamily (CYP3A). Inhibitors of P-glycoprotein are frequently inhibitors of CYP3A and vice-versa. See, for example, Wacher, V.J., et al., Overlapping substrate specificities and tissue distribution of cytochrome P4503A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy, Mol. Carcinogen, 13:129-134, 1995; Kim, R.B., et al., Interrelationship between substrates and inhibitors of human CYP3A and P-

glycoprotein, Pharm. Res., 16:408-44, 1999. Accordingly, with drugs such as quinidine, verapamil, valspodar, and cyclosporine A, a dual interaction occurs whereby reduced P-glycoprotein function is associated with increased plasma levels of the CYP3A substrate.

Although many P-glycoprotein inhibitors impair CYP3A-mediated metabolism, this is not an absolute relationship. In fact, the two characteristics appear to be independently determined such that some CYP3A inhibitors do not cause significant impairment of P-glycoprotein function and, more importantly, the reverse situation is possible, i.e., effective transporter inhibition with minimal effect on CYP3A. See Wandel, C., et al., P-glycoprotein and cytochrome P4503A inhibition: dissociation of inhibitory potencies, Cancer Res., in press, 1999. The 10,11-methanodibenzosuberanes of formula (I) are representative of such drugs. For example, the affinity of the compound of formula (II) for CYP3A is some 40-fold less than that for P-glycoprotein. Shepard, R.L., et al., Selectivity of the potent P-glycoprotein modulator, LY335979, Proc. Amer. Assoc. Cancer. Res., 39:362, 1998; Dantzig, A., J. Pharmco. Exp. Ther., 290:854-862, 1999. This selectivity would account for the relative small formula (II)-induced changes in nelfinavir's plasma level. Thus, the present invention has advantages over prior art P-glycoprotein inhibitors, since systemic toxicity from the antiviral agent would not be expected to increase following administration of compounds of formula (I).

An additional problem associated with prior art use of P-glycoprotein modulators has been their limited potency. Because of this limited potency, effective levels have been difficult to achieve without adverse effects. The minimal effects of quinidine, verapamil, ketoconazole, and cyclosporine A on nelfinavir's tissue:plasma ratios are consistent with such low potency as demonstrated by their IC_{50} values relative to digoxin translocation across Caco-2 cells. By contrast, the compound of formula (II) , which is at least 50-fold more potent than the other inhibitors, produced 75% to 90% inhibition of P-glycoprotein transport in both the brain and testes. This finding emphasizes the importance of potency in the application of P-glycoprotein modulators.

Another issue of selectivity by currently available P-glycoprotein modulators is related to the inhibition of P-glycoprotein itself versus other membrane

transporters that may also be involved in drug efflux or drug uptake into the cell. An increasing number of both types of membrane transporters have been identified and characterized in various cells/tissues within the body. Moreover, cross-inhibition of different transports appears to occur. For example, a number of P-glycoprotein inhibitors such as quinidine, verapamil, ketoconazole, and valspodar also impair drug uptake by OATP, but at higher concentrations than those required for inhibition of the efflux transporter. See Cvetkovic, M., et al., OATP and P-glycoprotein transporters mediate the coordinate cellular uptake and excretion of fexofenadine, Drug Metab. Disp., 27:866-871, 1999. Because an OATP type of transporter is present in the brain, (see Noe, B., Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain, Proc. Natl. Acad. Sci., 94:10346-10350, 1997), it is not unreasonable to suggest that the observed reduction in nelfinavir's plasma ratio with higher doses of cyclosporine A reflects such non-selectivity. A similar effort with valspodar has also been observed with another P-glycoprotein substrate - digoxin. In contrast, since the brain:plasma ratio continues to increase over the whole dose range studied, compound of formula (II) does not appear to inhibit transporters other than P-glycoprotein, at least in the brain. See Dantzig, supra.

Thus, the present invention employs the 10,11-methanodibenzosuberanes of formula (I) to increase HIV protease inhibitor concentrations in the brain and testes, without an associated increase in plasma concentrations.

The 10,11-methanodibenzosuberanes of formula (I) are typically co-administered with an HIV protease inhibitor, such as nelfinavir, saquinavir, indinavir, ritonavir, or amprenavir. In one preferred drug administration protocol a patient is pretreated with one or more doses of a compound of formula (I), and another dose of the P-glycoprotein inhibitor is administered concurrently with a dose of the HIV protease inhibitor. Typically, HIV protease inhibitors are administered orally in tablet form three times per day, in amounts of 600 to 1200 mg per dose. Administration of the compounds of formula (I) can be via any accepted mode of drug administration.

Dosage levels of the compound of formula (I) for use in accordance with this invention range can vary according to patient condition and weight but

generally range from about 0.01 to about 50 mg/kg of patient body weight, more preferably about 0.1 to 10 mg/kg of body weight, and most preferably about 0.3 to 2.0 mg/kg of body weight per dose.

The administration of the compounds of formula (I) in HIV treatment protocols with protease inhibitors in accordance with this invention can be carried out using any pharmaceutically acceptable mode of drug administration. The compounds of formula (I) can be administered either alone or more typically in combination with pharmaceutically acceptable excipients, including those used in formulating solid, semi-solid, liquid, or aerosol dosage forms, such as, for example, tablets, capsules, powders, liquids, suspensions, suppositories, nasal solutions, aerosols or the like. The compounds of formula (I) can also be administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, biodegradable matrices, transdermal (including electrotransport) patches, and the like, for the prolonged administration of the compound at a predetermined rate, preferably in unit dosage forms suitable for administration of precise dosages. The compositions will typically include a conventional pharmaceutical carrier or excipient and a compound of formula (I). In addition, the present compositions may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc., including a suitable dose of an HIV protease inhibitor. Generally, depending on the intended mode of administration, the pharmaceutically acceptable composition will contain about 0.1% to 90%, preferably about 0.5% to 50%, by weight of a compound or salt of formula (I), the reminder being suitable pharmaceutical excipients, carriers, etc.

One manner of administration of the compounds of formula (I) is oral, using a convenient daily dosage regimen which can be adjusted according to patient condition and total antiviral treatment protocol. For oral administration, a pharmaceutically acceptable composition is formulated by the combination of a compound of formula (I) and optional protease inhibitor with any of the normally employed pharmaceutical excipients, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium cross carmellose, glucose, gelatin, sucrose, magnesium carbonate, propylene carbonate, vegetable oils, or triglycerides, and the like. Such dosage compositions include solutions, suspensions, tablets, dispersible tablets, capsules, powders, lozenges, sustained release

formulations and the like. Preferably the compositions for oral administration will take the form of a tablet, capsule, or caplet.

Liquid pharmaceutical compositions in accordance with this invention can be prepared by dissolving, dispersing, etc. an active compound of formula (I) and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc.

Dosage forms or compositions containing active ingredient in the range of 0.005% to 95% with the balance made up from non-toxic carrier may be prepared. Other useful formulations include those set forth in U.S. Pat. Nos. Re. 28,819 and 4,358,603.

The present invention can also be carried out using formulations for parenteral administration, i.e, subcutaneous, intramuscular, intrathecal, or intravenous administration. Injectable dosage forms of this invention can be prepared as liquid solutions or suspensions, solid forms suitable for dissolution or suspension in liquid prior to injection, or as emulsions. Suitable excipient carriers are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, solubility enhancers, and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, cyclodextrins, etc. A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a more or less constant rate of drug release is maintained. See, e.g., U.S. Pat. No. 3,710,795.

The percentage of active compound contained in such parenteral compositions depends on the specific use and the needs of the subject. However, percentages of active ingredient of 0.01% to 10% in solution are acceptable, and they may be higher if the composition is a solid which will be subsequently diluted to the

above percentages. Preferably the composition will comprise 0.2 - 10% of the active agent in solution.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Example 1

Inhibition of the P-glycoprotein transport pump was measured as a function of inhibition of digoxin transport in an *in vitro* culture system. Inhibition of digoxin transport was determined using a polarized monolayer of Caco-2 cells. Caco-2 cells were grown and cultured on 0.4 μm polycarbonate membrane filters as described in Kim, R.B., et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest., 101:289-294, 1998. Transport of [^{3}H]-digoxin (15 Ci/mmol; Dupont-New England Nuclear, Boston, MA) was determined by its addition to either the basal or apical side of the polarized cell monolayer, and the transport over a four hour period of time of radioactivity into the other compartment was measured in the absence or presence of putative inhibitor in both compartments. The extent of inhibition by each putative inhibitor was determined using the following equation:

$$\% \text{ inhibition} = 1 - \left[\frac{i_{B-A} - i_{A-B}}{a_{B-A} - a_{A-B}}\right] \times 100$$

where i and a are the percentages of digoxin transport in the presence and absence of inhibitor, according to the direction of transport. IC_{50} values were estimated from the Hill equation using the computer program Prism® (GraphPad Software Inc., San Diego, CA), and the data represent results obtained from at least 3 preparations on different days.

IC_{50} values were calculated for various known P-glycoprotein inhibitors; ketoconazole (1 .2 μM), cyclosporine A (1.3 μM), verapamil (2.1 μM) and quinidine (2.2 μM), were in the low micromolar range. Fig. 1 illustrates the P-glycoprotein inhibition observed with various other putative inhibitors. Nelfinavir exhibited comparable inhibitory potency (1.4 μM) to the potency of the known P-glycoprotein inhibitors. However, ritonavir (3.8 μM) and saquinavir (6.5 μM) were somewhat less potent, and the IC_{50} value for indinavir (44μM) was about an order of magnitude greater than the IC_{50} values for the other HIV protease inhibitors. As shown in Fig. 1, the compound of formula (II) was by far the most potent of the P-glycoprotein inhibitors studied, with an IC_{50} value (0.024 μM) over 50-fold lower than cyclosporine A.

Example 2

The tissue distribution of nelfinavir in the absence of any other putative inhibitor was determined in *mdrla*(+/+) and *mdrla*(-/-) mice. Male *mdrla*(-/-) mice (FVB/TacfBR-[KO]mdrlaN7), 6-12 weeks of age and genetically matched male *mdrla*(+/+) mice (FVB/MTtacfBR) weighing 20 to 30 g were obtained from Taconic (Germantown, NY). The animals were cared for in accordance with the USPHS policy for the Care and Use of Laboratory Animals and the experimental studies were approved by the Vanderbilt University Animal Care Committee.

The tissue distribution of [^{14}C]-nelfinavir (8.5 mCi/mmol, Agouron Pharmaceuticals, Inc., San Diego, CA) was determined following intravenous injection (5 mg/kg) of an ethanol/0.9% saline solution over 5 minutes into a tail vein; the total volume injected was 4 μl/g. At specific times after drug administration and following anesthesia with isoflurane (Isoflo, Abbott Laboratories, Abbott Park, IL), blood was removed by orbital bleeding and the animal sacrificed. Subsequently, tissues were harvested, weighed, and homogenized with 4% bovine serum albumin solution. Total radioactivity was determined after the addition of 100 μl plasma or 500 μl tissue homogenate to vials containing 4 ml scintillation fluid (Scintiverse BD*, Fisher Scientific Co., Fairlawn, NJ). The brain:plasma ratio was 0.06 in the *mdrla*(+/+) mice, whereas the brain:plasma ratio was 2.3 in the *mdrla*(-/-) mice. The distribution also varied in the testes, where the *mdrla*(+/+) mice had a 0.29 testes:plasma ratio, and the *mdrla*(-/-) mice had a testes:plasma ratio of 2:1.

Example 3

The effect of P-glycoprotein inhibitors was investigated in *mdrla*(+/+) mice by pretreatment with equally divided doses given by intravenous tail vein injection, 30 minutes prior to and concurrently with administration of nelfinavir. Inhibitors studied included the compound of formula (II) (2 x 0.5 to 25 mg/kg, Lilly Research Laboratories, Indianapolis, IN), verapamil (2 x 6.25 mg/kg, Sigma-Aldrich, St. Louis, MO) and quinidine (2 x 25 mg/kg, Sigma-Aldrich), each dissolved in 20% ethanol/0.9% saline; cyclosporine A (2 x 0.5 to 25 mg/kg, Novartis Pharma AG, Basel, Switzerland) dissolved in 10% ethanol/60% propylene glycol/30% water; nelfinavir (2 x 25 mg/kg, Agouron Pharmaceuticals Inc., San Diego, CA), ritonavir (2 x 12.5 mg/kg Abbott Laboratories), saquinavir (2 x 25 mg/kg, Roche Products Ltd., Welwyn, UK), and indinavir (2 x 25 mg/kg, Merck Research Laboratories, West Point, PA) each dissolved in 10% ethanol/40% propylene glycol/50% 0.9% saline; and ketoconazole (2 x 25 mg/kg, Sigma-Aldrich) dissolved in 25% 0.2N HC1. All drugs were injected in total volume of 4 µl/g and appropriate vehicle solutions were used in control studies.

Similar tissue distribution studies were also performed to study tissue distribution of [^{14}C]-saquinavir (9.8 mCi/mmol, Roche Products Ltd) and [^{14}C]-indinavir (8.5 mCi/mmol. Merck Research Laboratories), using the compound of formula (II) (2 x 25 mg/kg) as the P-glycoprotein inhibitor.

At least 3 mice were studied at each time point and differences in radioactivity between treated and control groups were analyzed by a two-sided Student's t-test with $p < 0.05$ as the limit of statistical significance.

As shown in Fig. 3, pretreatment with 25 mg/kg formula (II), 30 minutes prior to and concurrently with [^{14}C]-nelfinavir, markedly altered the disposition of total radioactivity in *mdrla*(+/+) mice. The brain concentration-time profile in particular was especially affected, as seen in Fig. 2. In untreated mice, radioactivity in the brain was more than 17 times lower than that in plasma with a mean brain:plasma concentration ratio of 0.06, based on the relative area under the concentration-time curves. Formula (II) increased brain levels by 20-fold in contrast to those in the plasma, which only changed 2-fold. As a result, formula (II) treatment produced an 10-fold increase in nelfinavir's brain:plasma distribution ratio.

Subsequent studies, also illustrated in Fig. 3, based on tissue distribution measured two hours after nelfinavir administration showed that these changes are dose-dependent. Moreover, 10- to 15-fold higher brain levels could be achieved without affecting nelfinavir plasma concentrations at total dosages between 12.5 mg and 25 mg/kg. Comparison of these findings with those in *mdrla*(-/-) mice indicated that if all of the effects of formula (II) are accounted for by P-glycoprotein inhibition, then the transporter is inhibited by about 75% following a total dose of 50 mg/kg formula (II). Similar results were obtained with nelfinavir levels in the testes, with P-glycoprotein activity being inhibited by over 90%. Similar findings were also noted after intravenous administration of [^{14}C]-saquinavir, [^{14}C]-indinavir and pretreatment with 50 mg/kg formula (II).

More modest, though statistically significant changes, were produced by cyclosporine A, ketoconazole, and ritonavir administration, but these largely reflected increased nelfinavir plasma concentrations rather than altered tissue distribution. Finally, neither quinidine, verapamil, nelfinavir, saquinavir, or indinavir produced significant changes in nelfinavir's disposition at the doses studied. The results are summarized in Table 1:

Table 1: Tissue levels of radioactivity (ng/g tissue) in wildtype and *mdrla*(-/-) mice at 2 hr after intravenous injection of [^{14}C]-nelfinavir (5mg/kg). Mice were treated with varying doses of formula (II) or other known P-glycoprotein inhibitors, 50 mg/kg (unless otherwise noted) in two divided doses, given 30 min prior to and simultaneously with [^{14}C]-nelfinavir. Data are shown as mean ± standard deviation.

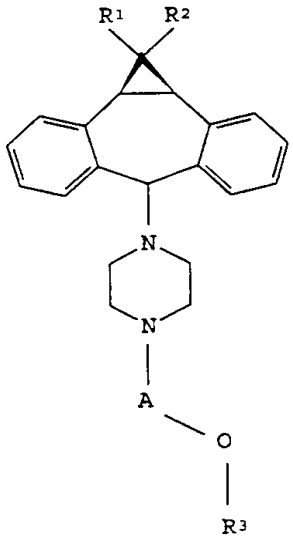
	Plasma	Brain	Brain:Plasma Ratio	Testes	Testes:Plasma Ratio
mdrla(+/+) Mice Vehicle Control	98 ± 12	5.1 ± 1.9	0.06 ± 0.01	31 ± 5.8	0.29 ± 0.02
Ritonavir (25 mg/kg)	618 ± 112	22 ± 3.8	0.08 ± 0.05	59 ± 3.4	0.31 ± 0.14
Nelfinavir (50 mg/kg)	124 ± 11	7.9 ± 1.5	0.06 ± 0.02	47 ± 6.6	0.39 ± 0.07
Saquinavir (50 mg/kg)	117 ± 14	6.9 ± 1.9	0.06 ± 0.01	48 ± 11	0.43 ± 0.13
Indinavir (50 mg/kg)	100 ± 6.2	7.5 ± 0.9	0.08 ± 0.01	37 ± 8.1	0.39 ± 0.05
Vehicle Control	99 ± 6.7	9.4 ± 3.0	0.10 ± 0.02	41 ± 6.8	0.38 ± 0.06
Quinidine (50 mg/kg)	92 ± 2.5	5.1 ± 1.5	0.06 ± 0.01	47 ± 7.3	0.54 ± 0.06
Verapamil (12.5 mg)	91 ± 6.1	8.4 ± 2.1	0.09 ± 0.02	39 ± 3.0	0.44 ± 0.06

	Plasma	Brain	Brain:Plasma Ratio	Testes	Testes:Plasma Ratio
Ketoconazole (50 mg/kg)	292 ± 68	57 ± 14	0.20 ± 0.02	87 ± 16	0.30 ± 0.04
Cyclosporine Vehicle Control	103 ± 13	9.2 ± 1.2	0.10 ± 0.03	66 ± 12	0.42 ± 0.04
1 mg/kg	120 ± 6.0	11 ± 2.4	0.10 ± 0.02	61 ± 7.0	0.51 ± 0.04
4 mg/kg	322 ± 14	45 ± 20	0.13 ± 0.06	128 ± 10	0.40 ± 0.05
12.5 mg/kg	698 ± 189	89 ± 19	0.18 ± 0.08	195 ± 54	0.30 ± 0.07
25 mg/kg	659 ± 57	190 ± 43	0.30 ± 0.08	294 ± 50	0.44 ± 0.04
50 mg/kg	954 ± 132	242 ± 46	0.27 ± 0.07	245 ± 55	0.25 ± 0.07
Formula (II) Vehicle Control	84 ± 4.9	6.6 ± 1.7	0.08 ± 0.02	47 ± 3.7	0.48 ± 0.07
1 mg/kg	74 ± 14	9.4 ± 1.7	0.11 ± 0.04	56 ± 1.7	0.81 ± 0.13
4 mg/kg	72 ± 4.8	24 ± 4.5	0.33 ± 0.04	95 ± 18	1.4 ± 0.33
12.5 mg/kg	71 ± 11	60 ± 5.4	0.89 ± 0.16	108 ± 27	1.6 ± 0.44
25 mg/kg	89 ± 8.1	89 ± 17	1.1 ± 0.28	168 ± 61	2.0 ± 0.48
50 mg/kg	171 ± 12	243 ± 19	1.4 ± 0.08	187 ± 17	1.2 ± 0.19
mdrla (-/-) Mice Vehicle	89 ± 15	184 ± 20	2.3 ± 0.24	194 ± 34	2.1 ± 0.35
Formula (II) (50mg/kg)	161 ± 24	318 ± 52	1.9 ± 0.12	207 ± 46	1.3 ± 0.13

Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

CLAIMS

1. A method for increasing the concentration of an HIV protease inhibitor in the brain of a patient, said method comprising administering to an HIV infected patient an amount of a 10,11-methanodibenzosuberane of formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof; and

co-administering to the patient a therapeutically effective amount of the protease inhibitor.

2. The method of claim 1 wherein the patient is a male and the concentration of the HIV protease inhibitor is also increased in the patient's testes.

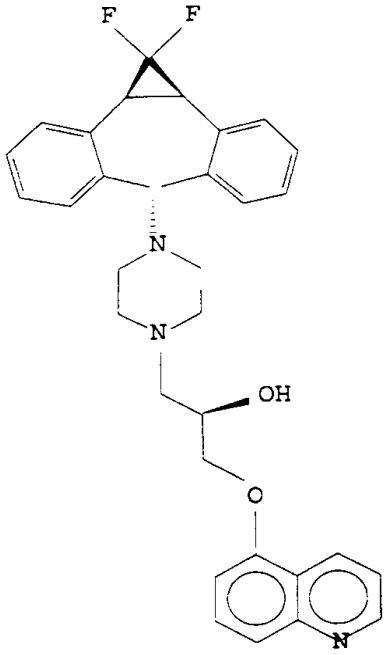
3. The method of claim 1 wherein the protease inhibitor is selected from the group of nelfinavir, indinavir, saquinavir, ritonavir, and amprenavir.

4. The method of claim 3 wherein the protease inhibitor is nelfinavir.

5. The method of claim 1 wherein R^1 and R^2 are F, A is $-CH_2CHR^aCH_2-$, and R^3 is optionally substituted quinolyl.

6. The method of claim 5 wherein R^a is OH and R^3 is quinol-5-yl.

7. The method of claim 1 wherein the methanodibenzosuberane of formula (I) is a compound of formula (II):

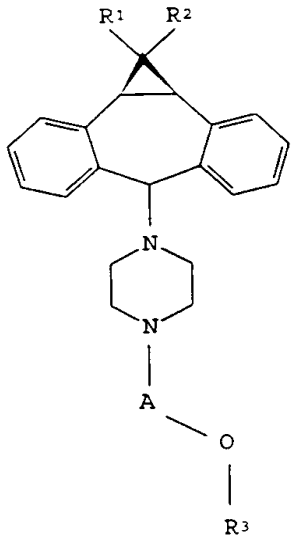


II

8. A method of treating a patient having an HIV-1 infection comprising:

administering to the patient a therapeutically effective amount of a protease inhibitor, and

co-administering to the patient an amount of a compound represented by formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof;

in an amount sufficient to increase brain levels of the protease inhibitor.

9. The method of claim 8 wherein R^1 and R^2 are F, A is $-CH_2CHR^aCH_2-$, and R^3 is optionally substituted quinolyl.

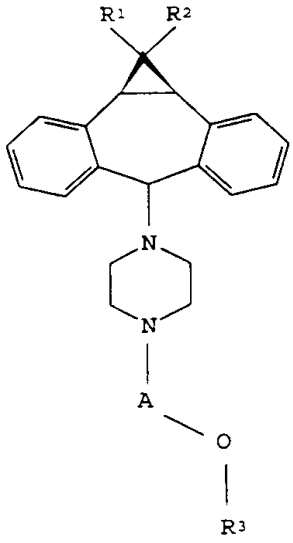
10. The method of claim 9 wherein R^a is OH and R^3 is quinol-5-yl.

11. The method of claim 8 wherein the amount of the compound of formula (I) is sufficient to increase the brain levels of the protease inhibitor without significantly increasing the concentration of the protease inhibitor in the patient's blood.

12. The method of claim 8, wherein the amount of the compound is also sufficient to increase concentrations of the protease inhibitor in the patient's testes.

13. A pharmaceutical composition comprising an antiviral protease inhibitor;

a 10,11-methanodibenzosuberane of formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof;

and a pharmaceutically acceptable carrier therefor.

14. The composition of claim 13 wherein the methanodibenzosuberane of formula (I) is present in an amount effective to increase brain levels of the protease inhibitor.

15. The composition of claim 14 wherein the methanodibenzosuberane of formula (I) is present in an amount effective to increase brain levels of the protease inhibitor without significantly increasing plasma levels of the protease inhibitor.

16. The composition of claim 13 wherein the protease inhibitor is selected from the group consisting of nelfinavir, indinavir, saquinavir, ritonavir, or amprenavir.

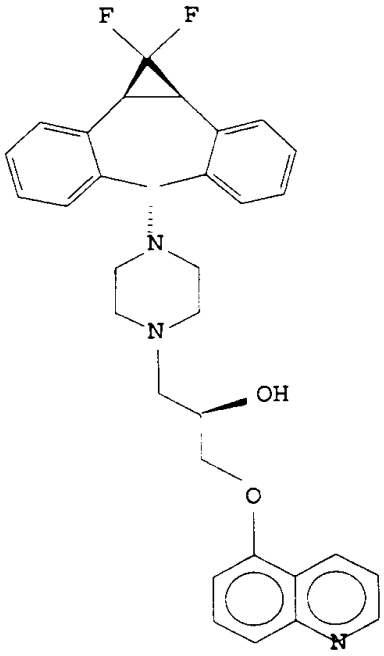
17. The composition of claim 16 wherein the protease inhibitor is nelfinavir.

18. The composition of claim 13 wherein R^1 and R^2 are F.

19. The composition of claim 13 wherein A is $-CH_2CHR^aCH_2-$.

20. The composition of claim 13 wherein R3 is a optionally substituted quinolyl.

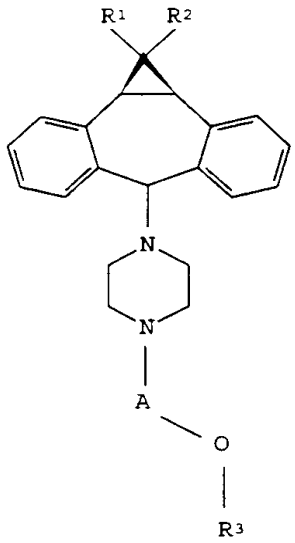
21. The composition of claim 13 wherein the 10,11-methanodibenzosuberane is the compound of formula (II):



II

22. The composition of claim 13 wherein the methanodibenzosuberane comprises about 0.005 to 95% of the composition.

23. Use of a 10,11-methanodibenzosuberane of formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof;

for the manufacture of a medicament for the treatment of HIV in a patient undergoing treatment with an HIV protease inhibitor.

24. The use of claim 23 for increasing the concentration of the protease inhibitor in the brain of a patient undergoing treatment with an HIV protease inhibitor.

25. The use of claim 24 for increasing the concentration of the protease inhibitor in the patient's testes.

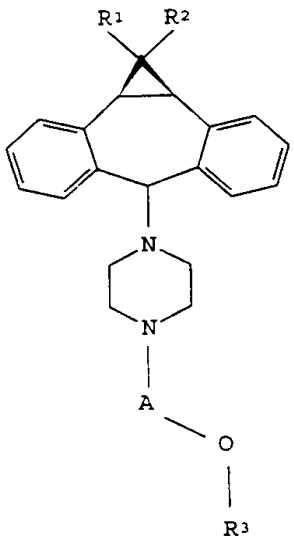
26. The use of any one of claims 23-25 for the manufacture of a medicament wherein the protease inhibitor is selected from the group of nelfinavir, indinavir, saquinavir, ritonavir, and amprenavir.

27. The use of any one of claims 23-26 for the manufacture of a medicament wherein R^1 and R^2 are F, A is $-CH_2CHR^aCH_2-$, and R^3 is optionally substituted quinolyl.

28. The use of claim 27 for the manufacture of a medicament wherein R^a is OH and R^3 is quinol-5-yl.

29. The use of claim 23 for the manufacture of a medicament for increasing brain levels of the protease inhibitor without significantly increasing plasma levels of the protease inhibitor.

30. Use of an HIV protease inhibitor for the manufacture of a medicament for the treatment of HIV wherein the concentration of HIV protease inhibitor in the brain is increased by co-administration with a 10,11-methanodibenzosuberane of formula (I):



I

wherein: A is $-CH_2CH_2-$; $-CH_2CHR^aCH_2-$ where R^a is H, OH or lower acyloxy; or $-CH_2CHR^bCHR^cCH_2-$ where one of R^b or R^c is H, OH, or lower acyloxy, and the other is H;

R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$; or a pharmaceutically acceptable salt thereof.

31. The use of claim 30 wherein the concentration of the protease inhibitor in the patient's testes is also increased.

32. The use of any one of claims 30-31 wherein the protease inhibitor is selected from the group of nelfinavir, indinavir, saquinavir, ritonavir, and amprenavir.

33. The use of claim 30 wherein the protease inhibitor is nelfinavir.

34. The use of any one of claims 30-33 wherein R^1 and R^2 are F, A is $-CH_2CHR^aCH_2-$, and R^3 is optionally substituted quinolyl.

35. The use of claim 34 wherein R^a is OH and R^3 is quinol-5-yl.

36. The use of claim 30 wherein the brain levels of the protease inhibitor are increased without significantly increasing plasma levels of the protease inhibitor.

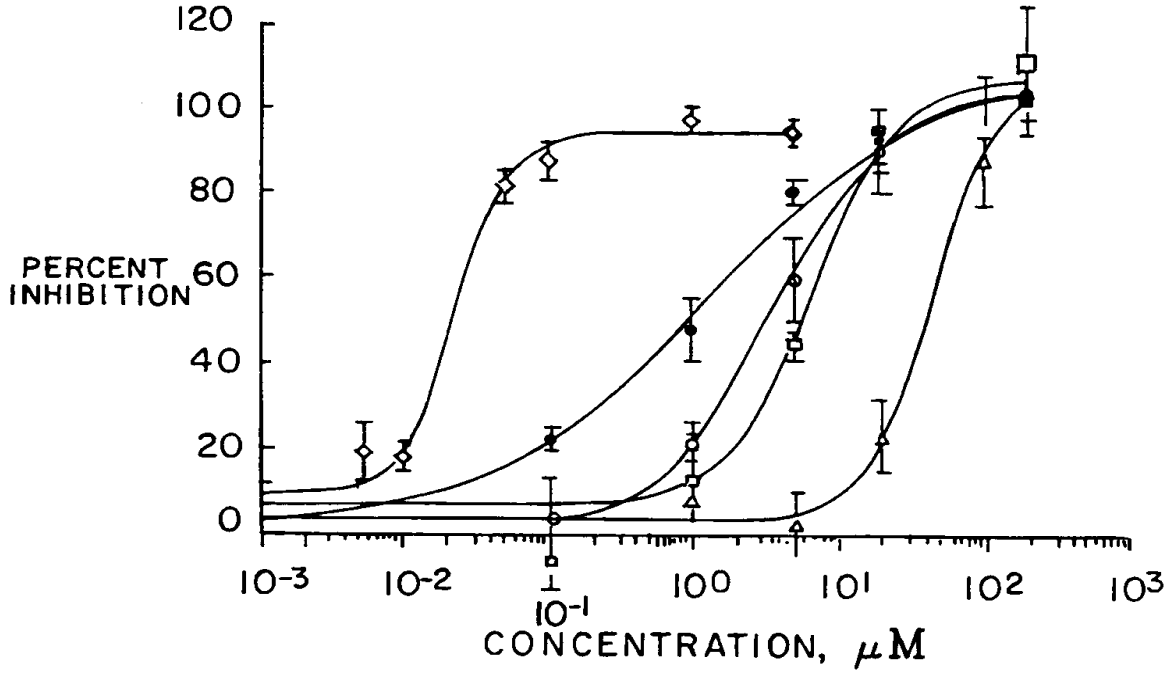

120
100
80
PERCENT INHIBITION
60
40
20
0
10-3
10-2
10-1
100
101
102
10³
CONCENTRATION, μM

FIG. 1

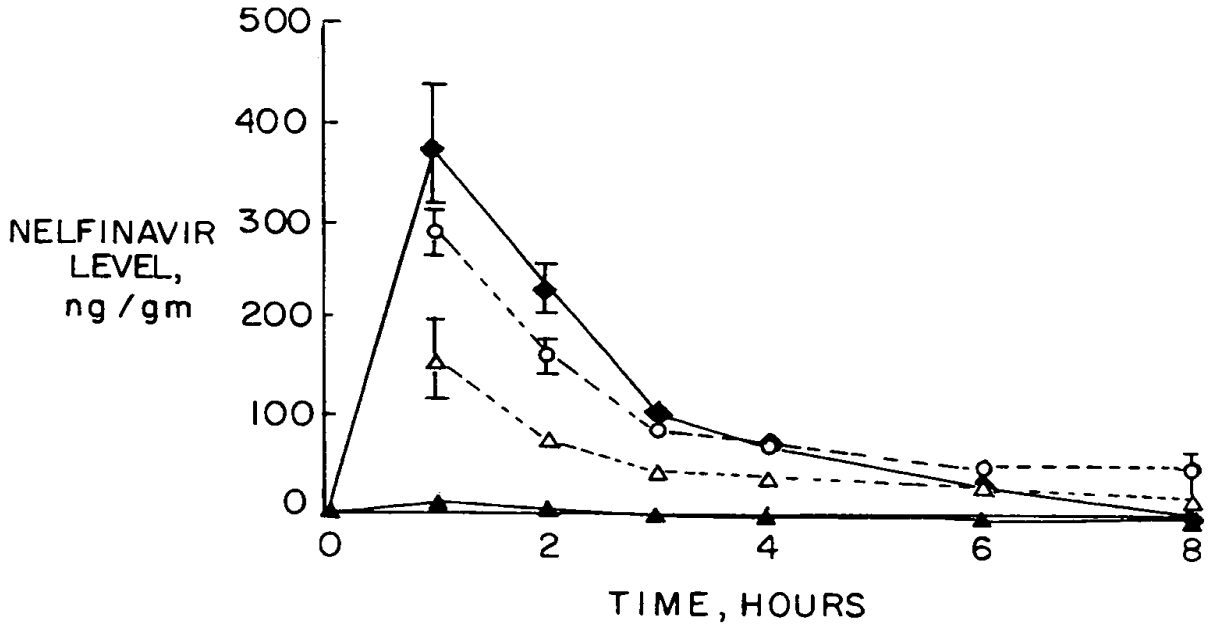

500
400
NELFINAVIR LEVEL, ng/gm
300
200
100
0
0
2
4
6
8
TIME, HOURS

FIG. 2

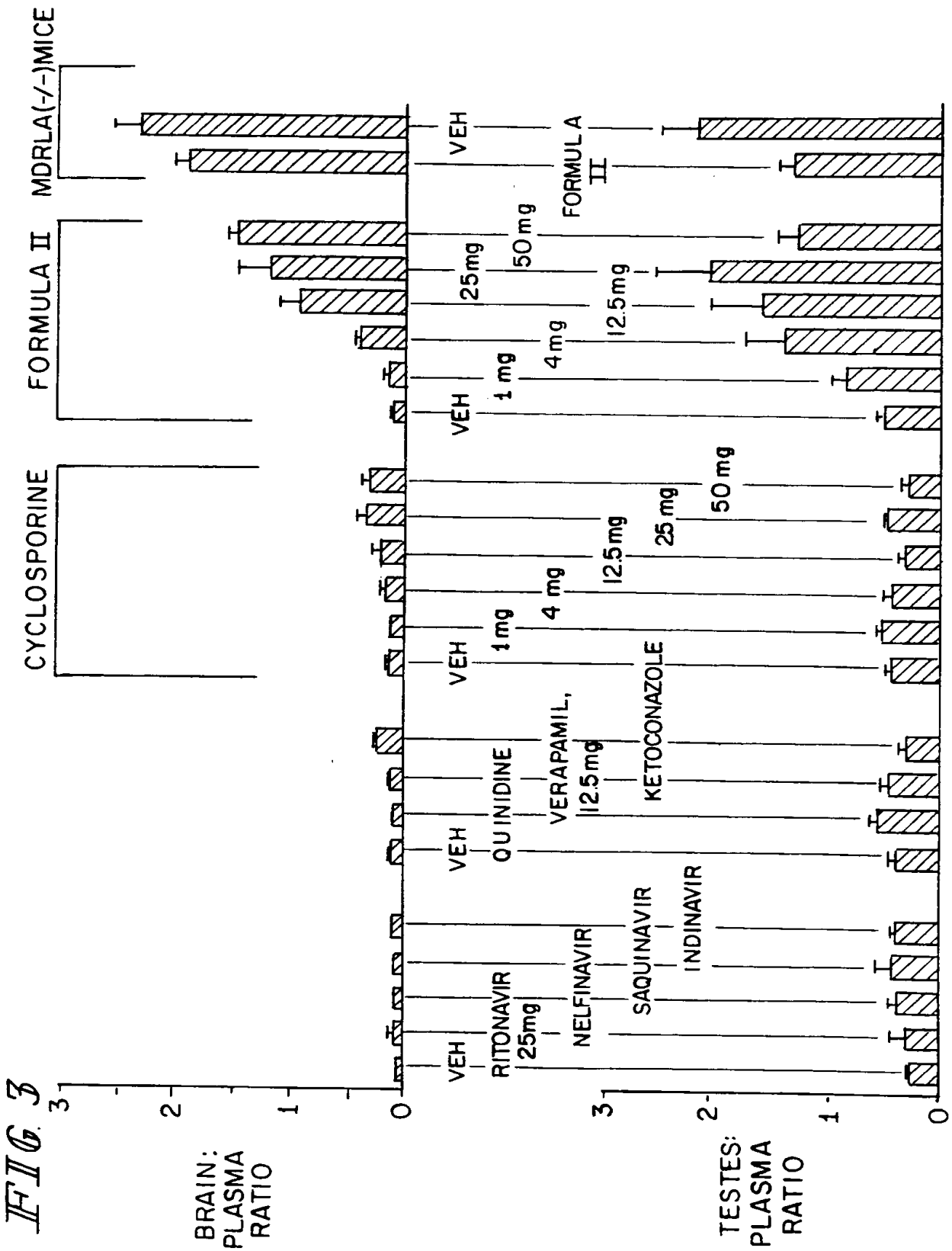

MDRLA(-/-)MICE
FORMULA II
CYCLOSPORINE
VEH
FORMULA II
1 mg
4 mg
12.5mg
25mg
50 mg
VEH
1 mg
4 mg
12.5mg
25 mg
50 mg
VEH
QUINIDINE
VERAPAMIL, 12.5mg
KETOCONAZOLE
VEH
RITONAVIR 25mg
NELFINAVIR
SAQUINAVIR
INDINAVIR
BRAIN: PLASMA RATIO
TESTES: PLASMA RATIO
0
1
2
3

FIG. 3